Studies on Vegetable Oils and Fats

(Acharya Jagadish Chandra Bose Memorial Lectures, 1986)

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I also gratefully acknowledge the inspiration received from Prof. N. R. Dhar another pathbreaker of Chemistry who has always encouraged me.

CONTENTS

Part I

RECENT STUDIES ON BIOGENESIS OF SEED FATS

INTRODUCTION :

I feel deeply honoured by being asked to deliver the first Acharya J. C. Bose Memorial Lectures instituted by Calcutta University. The contribution of Acharya Jagadish Chandra Bose as a person who placed India in the scientific map of the world after Ramanujam although well known needs to be told again to remind the present generation of what he did as a Research Physicist in his early days and later after he transformed himself as a Biophysicist who bridged the gulf between the living and non-living and excelled in both the areas of physics and biology. This emphasises the fact that the realm of science remains one. As a scholar, as a teacher, as a research guide and in his last phase as an administrator of research activities he proved that he could be the very best in his realm but above all as an initiator of research in students perhaps he will be remembered even more like his colleague Late Acharya P. C. Ray. Although I have been a student of Chemistry I have decided to pay my homage to the contribution of this great savant by presenting some aspects from the subject of lipids with which I have been connected both in teaching and research for the last three and a half decades. I have divided my presentation in three parts as (a) some studies on aspects of biogenesis of seed fats (b) the contribution of plant geneticists in changing the composition of oils and fats and (c) a discussion of industries based on fats and the developments that should be our concern in the scenario from industrial aspects up to 2000 A.D.

Before that I should like to give the definition of the word Lipids. This is a collective title for the whole group of natural products in which higher fatty acids are present as essential components. Natural fats from both animal and vegetable origin

constitute the most important group among the substances which are known as lipids. The first two tables (also in slides) give a description briefly of fats and compounds which coexists in fats or exist separately in plant and vegetable kingdom. I shall confine my discussion only briefly in this part to the topic of biogenesis of fats in the plant. But before that it is necessary to state that the fats are esters of fatty acids of carbon chain length from C_4 to C_{26} with glycerol as the alcohol.

The Tables (Slides 1—6) show the glycerol and the fatty acids that are generally combined in natural fats and a general formula of a triglyceride, the chemical name of fats.

Fats and oils are indispensable item of consumption in our life catering to our physiological requirements by providing energy and other biologically active lipid derivatives.

Fats and oils are available from plant and animal origins. About 80 per cent of the total oils and fats that are produced in the world come from vegetable sources mainly plant seeds and tree borne seeds and from other vegetable oil-bearing materials.

The formation of fats in oil-bearing materials have some unique biogenetic features. Fats which are stored in oilseeds are converted into carbohydrates during germination. Fats are basically triglycerides and these triglycerides are hydrolised by lipases and the fatty acids that are released become oxidised to acetyl-CoA. Glucose is ultimately synthesized from this acetyl-CoA. The conversion of fat into carbohydrate is well established and the carbohydrates are again converted to fats when seeds are formed with the growth of plants. The proteins which also occur in seeds act as possible progenitors of fats. In the extensive studies on the biogenesis of fats in plants, observations are recorded to indicate that with the ripening of seeds the percentage of carbohydrates decreases and that of protein also decreases while the content of fat increases till a stage is reached when a matured seed contains, in general, oil as major component and carbohydrates and protein as the minor components. In fact, there is a rapid cycle about conversion of carbohydrate into fat which varies in time period for different seeds. The investigations on seeds[1] like almond, olive, poppy, linseed, cottonseed, niger seed, cocoabean and tung seed etc. exemplify convincingly

the interrelationship of fat, carbohydrate and protein metabolism in plant seeds (Slide 7-9).[1]

The most interesting part of the biogenesis includes the synthesis of various fatty acids that occur in oils and fats in the form of triglycerides and other lipid derivatives. There are two main aspects on the synthesis of fatty acids in the seed oils. One such aspect deals with the percentage of the formation of various fatty acids from the stage of flowering to the stages of seed maturation. The published information reveal that all the unsaturated acids are produced at very similar rates throughout. There could be, however, difference in the ratio of the various unsaturated acids in different seeds with respect to the time of seed maturation. It is, however, an established fact that the oleic acid in most cases is produced more rapidly but afterwards more polyenoic unsaturated acid like linoleic and linolenic become the major components when all the three acids occur in the seed oil with linoleic and/or linolenic as the major unsaturated fatty acids in the matured seed oils (Slides 10-12).[1]

The effect of agronomical factors such as temperature on the synthesis of fatty acids is also an interesting phenomenon. It has been observed[3] that in cooler conditions the seed fats contain more unsaturated fatty acids and less oleic acid. There is, however, a very short increase of saturated acids in the most unsaturated seed oils (Slide 13).

Saturated acids in the seed fats reveal, however, that one acid is generally predominant and it is invariably accompanied by smaller proportions of the next higher or lower or usually both saturated acids.

In nature, the occurrence of seed fats having oxygenated fatty acid are only very few. The well known oxygenated fatty acid rich oil is castor oil which contains about 85 per cent hydroxy oleic acid with hydroxyl group being attached to the 12th carbon atom. Its biosynthesis is preceded by the synthesis of oleic acid followed by hydroxylation at the 12th carbon atom.

Another oxygenated fatty acid occurs in 70 to 80 per cent in the plant seed of *Vernonia anthelmintica*. Its biogenesis has been studied by Miwa et al.[4] at the different stages of formation

of seeds. It has been observed that in the early stages of seed maturation oleic acid is a major component which is gradually converted to C(12 : 13) dihydroxy oleic acid. This dihydroxy acid increases until the full maturity of seeds is approached when it declines markedly with concommitant increase of epoxy oleic acid. The biosynthetic sequence[4] of oleic acid is therefore oleic→12-13 dihydroxy oleic→epoxy oleic acid (Slide 14).

Cyclopropene fatty acids form a class of unusual acid present in plant species belonging to Sterculiaceae, Bombacaceae and Malvacae. Only two cyclopropene fatty acids are known to occur in nature, namely, malvalic acid (18 : 1 cyclopropene fatty aid) and sterculic acid (19 : 1—cyclopropene fatty acid). The biosynthesis of these two acids[5, 6] is indicated in (Slide 15).

The second and the most important part of biogenesis is the study of the mechanism of biosynthesis of the various saturated and unsaturated fatty acids. The exhaustive investigations indicate that the enzymes are involved in the various stages of fatty acid synthesis. In the case of normal saturated acids two distinct pathways are recognised. One leads to the synthesis of fatty acid from acetate C_2 precursors while the other involves elongation of a preformed fatty acid by a C_2 unit.

Recent investigations by Stumpf[7] have shown : (a) the synthesis of palmitic, stearic and oleic acids occur exclusively in chloroplasts of the leaf cell or in proplastids of the seed and mesocarp cells (b) the enzymes involved in these syntheses are all nonassociated and strongly resemble a simple fatty acid synthase system found in procaryotic cells.

Enzymes located in the cytoplasm of the cell catalyse the conversion of acetyl—CoA to long chain acids especially palmitic in the presence of (Adenosine Triphosphate) ATP, Mn^{++}, HCO_3^-—and (reduced Nicotinamide adenosine dinucleotide phosphate) NADPH. The condensation leads, via a series of reactions, to palmitic acid accompanied by smaller amounts of lauric, myristic or stearic acid. The over all reaction[8] has been represented (Slide 16) :

$CH_3CO.SCoA + 7HO_2C.CH_2.CO.SCoA + 14NADPH + 14H^+ \rightarrow$

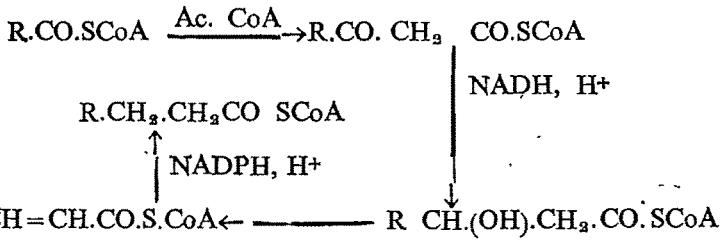
Acetyl Co-enzyme A — Malonyl — CoA — Reduced Nicotinamide adenosine dinucleotide phosphate

$CH_3.(CH_2)_{14}.CO.S.CoA + 14NADP^+ + 7CO_2 + 7CoASH + 7H_2O$

Palmityl CoA — Nicotinamide adenosine dinucleotide phosphate

(c) Modification of the palmitic acid and stearic acid hydrocarbon chain occur in other compartments, notably the endoplasmic reticulum compartment where desaturations, elongations, or hydroxylations as well as triacylglycerol or phospholipid synthesis occurs.

The overall reaction is[6] (Slide 17) :



(d) two key enzymes[7] -keto acyl (acetyl carrier protein) ACP synthetase I and -keto acyl synthetase II play key roles in regulating palmitic acid and stearic acid synthesis. Synthetase I is involved in synthesis from $C_2 \rightarrow C_{16}$ and synthetase II from $C_{16} \rightarrow C_{18}$.

(e) the synthesis of very long chain fatty acids occurs on membrane systems in the cytosol of the cell. Their synthesis is not related to the C_2—C_{18} system but involves an entirely different set of enzymes although the chemistry is the same.

Though more than eighty monoethenoid acids have been recognised only a few occur commonly. They include oleic

acid followed by 16 : 1 (9C), 18 : 1 (11C), 20 : 1 (9C and 11C), and 22 : 1 (11C and 13C). Three different pathways have been recognized, but, once formed, monoenoic acids can be elongated by two-carbon units and it is likely that the acids named above are interrelated in the following way and that this extension process continues to other less common acids :

$$16:1\ (9C) \longrightarrow 18:1\ (11C)$$
$$18:1\ (9C) \longrightarrow 20:1\ (11C) \longrightarrow 22:1\ (13C)$$
$$20:1\ (9C) \longrightarrow 22:1\ (11C)$$

This elongation, like that already described for saturated acids, involves interaction of long-chain, acyl-coenzyme A derivatives, with acetyl-CoA or, in some cases, malonyl CoA.

In plants the polyunsaturated acids are mainly C_{18} acids. Plants contain enzymes capable of introducing double bonds between an existing double bond and the ω-methyl group and thus generate linoleic acid from oleic acid, linolenic acid from linoleic. These unsaturated centres[6] have 18 : 1 (9C)→18 : 2 (9C, 11C)→18 : 3 (9C, 12C, 15C) cis-configuration and are separated from one another by a single CH_2 group. The desaturation process is oxygen dependent, but little is known about the nature of possible intermediates. Ricinoleic acid seems an obvious choice, but there is evidence against the reaction sequence :

$$18:1\ (9C) \longrightarrow 12\text{-OH}\ 18:1\ (9C) \longrightarrow 18:2\ (9C,12C).$$

Conjugated polyolefinic acids are probably derived[8] mainly from linoleic acid by (i) hydroxylation followed by dehydration or (ii) epoxidation followed by a novel rearrangement and then dehydration as shown in Tables (Slides 18-19). The conversion of linoleic acid to conjugated trienoic acids thus proceeds via known natural compounds. By either scheme, linoleic acid would be the precursor for parinaric acid. Linoleic, Coronaric, α-dimorphecolic crepenynic and helenynolic acid, all of which co-exist in *Helichrysum bracteatum* seed oil.

The biogenesis of acetylenic fatty acids can be seen from Slide XIX. It involves epoxidation and rearrangement. Other mechanisms suggest the elimination and decarboxylation of enol pyrophosphates and also further desaturation of olefinic acids.

CONCLUDED PART :

The generalisations that emerge may be summarised as follows :—

1. Unsaturated seed fatty acids are built up by an entirely different mechanism from that which operates in the synthesis of saturated acids.

2. Each species of plant elaborates its own specifc mixture of acids in its seed fat ; but in seeds of the same species the relative amounts of oleic and linoleic (or linolenic) acids may vary considerably, such variation being conditioned mainly by the temperature of the locality where the seed ripens. The most unsaturated member of the group is found most abundantly in seeds grown in cool conditions, i.e., where the rate of synthesis is relatively slow. This suggests that the most unsaturated members (e.g. linolenic or linoleic), or their immediate precursors, are formed before (or, as it were, on the way to) the monoethenoid acid. The order of synthesis of unsaturated acids may be presumed, in the light of present knowledge, to be trie-ene (or, more probably, precursor)—diene (or, more probably, precursor)—mono-ene (oleic in the case of C_{18} acids).

3. Seed fat saturated acids differ from the unsaturated group in that, whilst one acid (usually palmitic) predominates, it is invariably accompanied by smaller proportions of the next higher, or lower, even-numbered homologue (usually by both). It would appear further that the biosynthesis in seeds of saturated acids is not quite so greatly affected by temperature.

TABLE I SATURATED ACIDS, $C_nH_{2n}O_2$ or C_mH_{2m+1}. COOH[1]

MOLECULAR FORMULA	COMMON NAME	SYSTEMATIC NAME	STRUCTURAL FORMULA
$C_4H_8O_2$	Butyric	n-Butanoic	$CH_3.(CH_2)_2.COOH$
$C_5H_{10}O_2$	iso-Valeric	3-Methy1-n-butanoic	$(CH_3)_2.CH.CH_2.COOH$
$C_6H_{12}O_2$	Caproic	n-Hexanoic	$CH_3.(CH_2)_4.COOH$
$C_8H_{16}O_2$	Caprylic	n-Octanoic	$CH_3.(CH_2)_6.COOH$
$C_{10}H_{20}O_2$	Capric	n-Decanoic	$CH_3.(CH_2)_8.COOH$
$C_{12}H_{24}O_2$	Lauric	n-Dodecanoic	$CH_3.(CH_2)_{10}.COOH$
$C_{14}H_{28}O_2$	Myristic	n-Tetradecanoic	$CH_3.(CH_2)_{12}.COOH$
$C_{16}H_{32}O_2$	Palmitic	n-Hexadecanoic	$CH_3.(CH_2)_{14}.COOH$
$C_{18}H_{36}O_2$	Stearic	n-Octadecanoic	$CH_3.(CH_2)_{16}.COOH$
$C_{20}H_{40}O_2$	Arachidic	n-Eicosanoic	$CH_3.(CH_2)_{18}.COOH$
$C_{22}H_{44}O_2$	Behenic	n-Docosanoic	$CH_3.(CH_2)_{20}.COOH$
$C_{24}H_{48}O_2$	Lignoceric	n-Tetracosanoic	$CH_3.(CH_2)_{22}.COOH$
$C_{26}H_{52}O_2$	'Cerotic'	n-Hexacosanoic	$CH_3.(CH_2)_{24}.COOH$

TABLE II

UNSATURATED ACIDS[1]

Mono-ethenoid acids, $C_nH_{2n-2}O_2$ or $C_mH_{2m-1}.COOH$

MOLECULAR FORMULA	COMMON NAME	SYSTEMATIC NAME	STRUCTURAL FORMULA
$C_{10}H_{18}O_2$		Dec-9-enoic	$CH_2 : CH.(CH_2)_7.COOH$
$C_{12}H_{22}O_2$		Dodec-9-enoic	$CH_3.CH_2.CH : CH.(CH_2)_7.COOH$
$C_{14}H_{26}O_2$		Tetradec-5-enoic	$CH_3.(CH_2)_7.CH : CH.(CH_2)_3.COOH$
$C_{14}H_{26}O_2$		Tetradec-9-enoic	$CH_3.(CH_2)_3.CH : CH.(CH_2)_7.COOH$
$C_{16}H_{30}O_2$	Palmitoleic, zoomaric	Hexadec-9-enoic	$CH_3.(CH_2)_5.CH : CH.(CH_2)_7.COOH$
$C_{18}H_{34}O_2$	Oleic	Octadec-9-enoic	$CH_3.(CH_2)_7.CH : CH.(CH_2)_7.COOH$
$C_{18}H_{34}O_2$	Petroselinc	Octadec-6-enoic	$CH_3.(CH_2)_{10}.CH : CH.(CH_2)_4.COOH$
$C_{18}H_{34}O_3$	Ricinoleic	12-hydroxy-octadec-9-enoic	$CH_3.(CH_2)_5.CH(OH).CH_2CH : CH.(CH_2)_7.COOH$
$C_{18}H_{34}O_3$		9-Hydroxy-octadec-12-enoic	$CH_3.(CH_2)_4.CH : CH.(CH_2)_2.CH(OH).(CH_2)_7.COOH$
$C_{18}H_{32}O_3$	Vernolic	12, 13-Epoxy-octadec-9-enoic	$CH_3.(CH_2)_4.CH.CH.CH_2.CH:CH.(CH_2)_7.COOH$ (O bridge)
$C_{19}H_{34}O_2$	Sterculic	8-(2-n-octylcycloprop-1-enyl) octanoic	$CH_3.(CH_2)_7.C:C.(CH_2)_7.COOH$ (CH_2 bridge)

TABLE II (Contd.)

$C_{20}H_{38}O_2$	Gadoleic	Eicos-9-enoic	$CH_3.(CH_2)_9.CH:CH.(CH_2)_7.COOH$
$C_{20}H_{38}O_2$		Eicos-11-enoic	$CH_3.(CH_2)_7.CH:CH.(CH_2)_9.COOH$
$C_{22}H_{42}O_2$	Cetoleic	Docos-11-enoic	$CH_3.(CH_2)_9.CH:CH.(CH_2)_9.COOH$
$C_{22}H_{42}O_2$	Erucic	Docos-13-enoic	$CH_3.(CH_2)_7.CH:CH.(CH_2)_{11}.COOH$
$C_{24}H_{46}O_2$	Selacholeic, nervonic	Tetracos-15-enoic	$CH_3.(CH_2)_7.CH:CH.(CH_2)_{13}.COOH$
$C_{26}H_{50}O_2$	Ximenic	Hexacos-17-enoic	$CH_3.(CH_2)_7.CH:CH.(CH_2)_{15}.COOH$
$C_{30}H_{58}O_2$	Lumequic	Triacont-21-enoic	$CH_3.(CH_2)_7.CH:CH.(CH_2)_{19}.COOH$
		Mono-ethynoid acid, $C_nH_{2n-4}O_2$ or $C_mH_{2m-3}.COOH$	
$C_{18}H_{32}O_2$	Tariric	Octadec-6-enoic	$CH_3.(CH_2)_{10}.CH:CH.(CH_2)_4.COOH$

TABLE III

Mono-ethenoid-diethynoid acid, $C_nH_{2n-10}O_2$ or C_mH_{2m-9}. $COOH$[1]

$C_{18}H_{26}O_2$	Isanic, erythrogenic	Octadec-17-en-9,11-di-ynoic	$CH_2 : CH.\ (CH_2)_4.\ C{:}C.\ C{:}C.\ (CH_2)_7.\ COOH$

Cyclic unsaturated acids, $C_nH_{2n-4}O_2$ or C_mH_{2m-3}. $COOH$

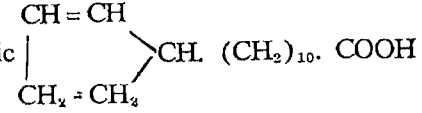
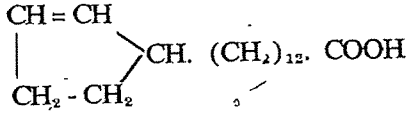
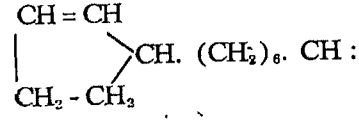
$C_{16}H_{28}O_2$	Hydnocarpic	11-cycloPent-2-enyl-n-undecanoic	
$C_{18}H_{32}O_2$	Chaulmoogric	13-cycloPent-2-enyl-n-tridecanoic	
$C_{18}H_{30}O_2$	Gorlic	13-cycloPent-2-enyl- n-tridec-6-enoic	

TABLE IV

Diethenoid acids, $C_nH_{2n-4}O_2$ or C_mH_{2m-3}. COOH[1]

$C_{18}H_{32}O_2$	Linoleic	Octadeca-9, 12-dienoic	CH_3. $(CH_2)_4$. CH : CH. CH_2. CH : CH. $(CH_2)_7$. COOH

Tri-ethenoid acids, $C_nH_{2n-6}O_2$ or C_mH_{2m-5}. COOH

$C_{16}H_{26}O_2$	Hiragonic	Hexadeca-6, 10, 14-trienoic	CH_3. CH : CH. CH_2. CH_2. CH : CH. $(CH_2)_2$CH : CH. $(CH_2)_4$. COOH
$C_{18}H_{30}O_2$	Linolenic	Octadeca-9, 12, 15-trienoic	CH_3. CH_2. CH : CH. CH_2. CH : CH. CH_2CH : CH. $(CH_2)_7$. COOH
$C_{18}H_{30}O_2$		Octadeca-6, 9, 12-trienoic	CH_3. $(CH_2)_4$. CH : CH. CH_2. CH : CH. CH_2. CH : CH. $(CH_2)_4$. COOH
$C_{18}H_{30}O_2$	Elaeostearic	Octadeca-9, 11, 13-trienoic	CH_3. $(CH_2)_3$. CH : CH. CH : CH. CH : CH. $(CH_2)_7$. COOH
$C_{18}H_{30}O_2$	Licanic	4-Keto-octadeca-9, 11, 13-trienoic	CH_3. $(CH_2)_3$. CH : CH. CH : CH. CH : CH. $(CH_2)_4$. CO. $(CH_2)_2$. COOH

TABLE V — Poly-ethenoid acids[1]

		(i) TETRA-ETHENOID
$C_{16}H_{24}O_2$		Hexadecatetraenoic
$C_{18}H_{28}O_2$	Parinaric	Octadeca-9, 11, 13, 15-tetraenoic $CH_3.\ CH_2.\ (CH:CH)_4.\ (CH_2)_7.\ COOH$
$C_{18}H_{28}O_2$	Stearidonic	Octadecatetraenoic
$C_{20}H_{32}O_2$	Arachidonic	Eicosa-5, 8, 11, 14-tetraenoic $CH_3.\ (CH_2)_4.\ CH:CH.\ CH_2.\ CH:CH.\ CH_2CH:CH.\ CH_2.\ CH:CH.\ (CH_2)_3.\ COOH$
(?)$C_{22}H_{36}O_2$		Docosatetraenoic
		(ii) PENTA-ETHENOID
$C_{20}H_{30}O_2$		Eicosapentaenoic
$C_{22}H_{34}O_2$	'Clupanodonic'	Docosapentaenoic
$C_{26}H_{42}O_2$	Shibic	Hexacosapentaenoic
		(iii) HEXA-ETHENOID
$C_{22}H_{32}O_2$		Docosahexaenoic
$C_{24}H_{36}O_2$	Nisinic	Tetracosahexaenoic
$C_{26}H_{40}O_2$	Thynnic	Hexacosahexaenoic

TABLE VI Triglyceride*

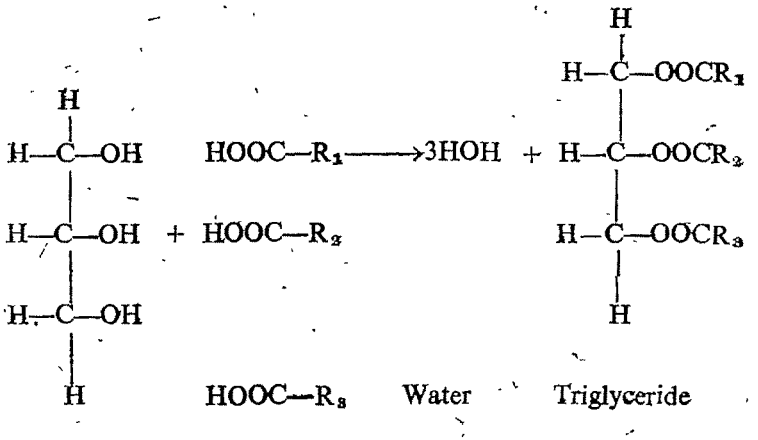


TABLE VII : Percentage contents of carbohydrates and fat in Almonds during ripening[1]

	June 9	July 4	August 1	Sept. 1	Oct. 4
Glucose%	6·0	4·2	0	0	0
Sucrose%	6·7	4·9	2·8	2·6	2·5
Starch%	21·6	14·1	6·2	5·4	5·3
Fat%	2·0	10·0	37·0	44·0	46·0

TABLE VIII : Percentage contents of carbohydrates and fat in Walnuts during ripening[1]

	July 6	August 1	August 15	Sept. 1	Oct. 4
Glucose%	7·6	2·4	0	0	0
Sucrose%	0	0·5	0·6	0·8	1·6
Fat%	3·0	16·0	49·0	52·0	62·0

TABLE IX : Percentage contents of protein and fat in Olive during ripening[1]

	August 30	September 30
Protein%	14·6	4·2
Fat%	29·2	62·3

TABLE X : (i) Fats and carbohydrates in ripening cotton seed[1]

Percentage composition			Composition of fatty acids		
Age of seeds (days)	Carbohydrate	Total lipids	Saturated	Oleic	Linoleic
21	75·7	2·2	—	—	—
31	75·7	2·4	23·9	29·3	46·8
41	61·5	10·3	22·9	26·4	50·7
51	35·3	21·7	20·5	27·7	51·8
60	30·3	25·3	22·4	25·5	52·1

(ii) Weights of components in 100 seeds (bolls)

Age of seeds	Carbohydrate	Total lipids	Individual fatty acids (GM)		
(days)	(GM)	(GM)	Saturated	Oleic	Linoleic
21	25·1	0·7	—	—	—
31	62·8	2·0	0·3	0·4	0·6
41	91·3	15·3	3·3	3·8	7·3
51	84·4	51·8	10·1	13 6	25·5
60	67·9	56·9	12·0	13·7	28·0

TABLE X (contd.) (iii) Free fatty acids and unsaponifiable matter in total lipids

Age of seeds (days)	Free fatty acids		Unsaponifiable matter	
	Percent of lipids	Wt. (GM) in 100 seeds	Percent of lipids	Wt. (GM) in 100 seeds
21	12. 9	0. 09	41. 5	0. 30
31	12. 9	0. 25	26. 7	0. 52
41	2. 5	0. 38	2. 7	0. 41
51	1. 3	0. 66	1. 7	0. 86
60	1. 1	0. 62	1. 3	0. 74

TABLE XI Development of fat in ripening linseed[1]

	Days after flowering			
	10	20	30	40
Linseed (RAJA, 1956) 100 seeds (wt., gm.)	1.00	0.99	0.91	0.79
Oil content (%, dry wt.)	4.9	33.3	39.8	38.5
Oil, Iodine value	122.9	172.8	180.8	181.0
Component acids (mgm. per 100 seeds):				
Oleic	3.6	31.9	40.9	60.7
Linoleic	2.1	27.3	46.7	51.8
Linolenic	2.2	60.9	110.3	118.4

TABLE XII Development of fat in ripening Safflower[1]

	Days after flowering			
	20	30	40	50
Safflower (INDIAN, 1957)				
100 seeds (wt., gm.)	4.80	4.77	4.48	4.42
Oil content (percent, dry wt.)	15.0	20.2	21.9	22.1
Oil, iodine value	138.3	136.3	144.1	146.2
Component acids (mgm. per 100 seeds) :				
Oleic	114.9	151.4	140.5	148.8
Linoleic	297.7	453.8	571.1	602.4
(Ratio linoleic/Oleic)	2.6	3.0	4.1	4.0

TABLE XIII Fatty acid composition of Safflower Types Grown Under Different Temperature in 1970[3]

Type	Temperature*	Fatty acid , %			
		Palmitic	Stearic	Oleic	Linoleic
High linoleic	Low	6.5	1.9	9.1	82.4
	Intermediate	6.4	1.4	14.0	78.2
	High	7.3	2.0	15.2	75.5
High oleic	Low	5.4	0.9	69.7	24.0
	Intermediate	5.2	1.8	71.6	21.4
	High	6.0	2.3	77.4	14.3
Intermediate linoleic	Low	6.5	1.9	16.5	75.1
	Intermediate	6.7	0.8	29.0	63.5
	High	6.5	1.8	53.1	38.6
High stearic	Low	6.1	4.3	9.0	80.6
	Intermediate	6.7	5.5	10.9	76.9
	High	6.5	10.6	14.2	68.7

* Day and night temperatures were respectively as follows :— Low — 18.4, 15.6°C; Intermediate — 26.7, 23.9°C and High — 29.4, 21.1°C

TABLE XIV Biosynthetic Pathway of Epoxyoleic Acid[4]

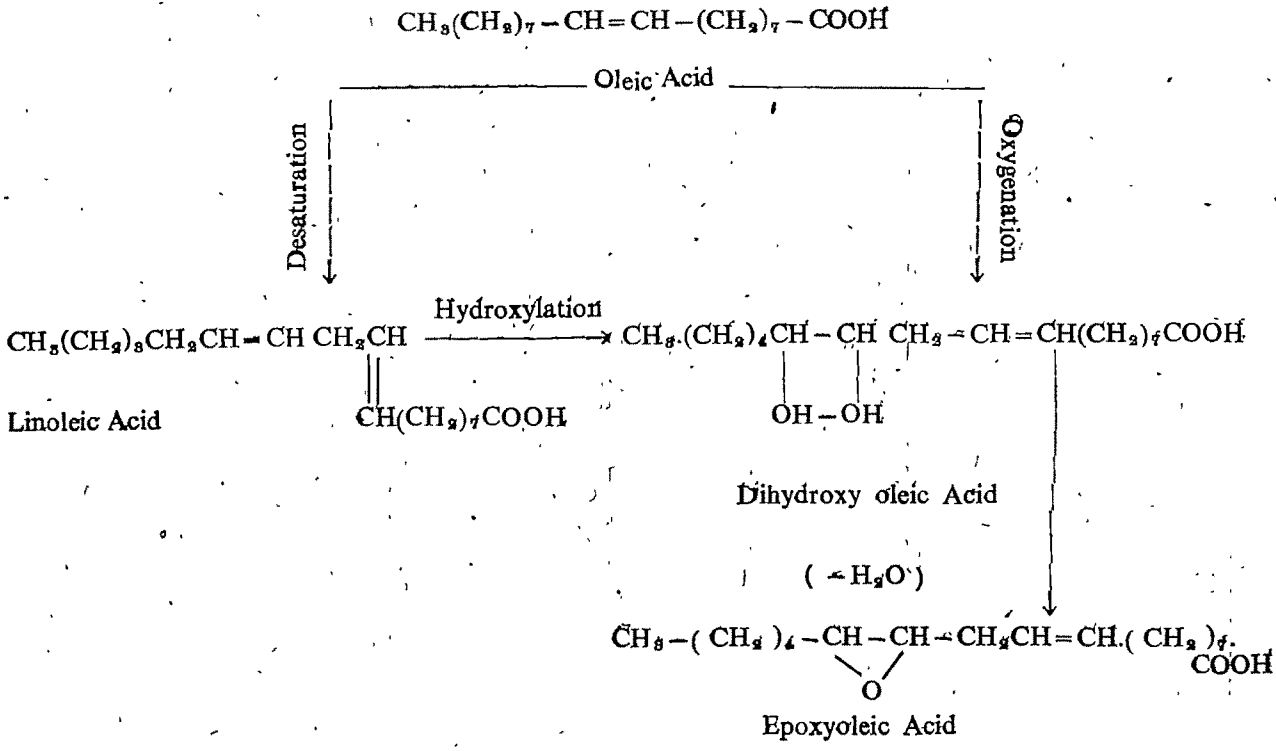


TABLE XV

Biosynthetic Pathways of Cyclopropene Fatty Acids (CFA)

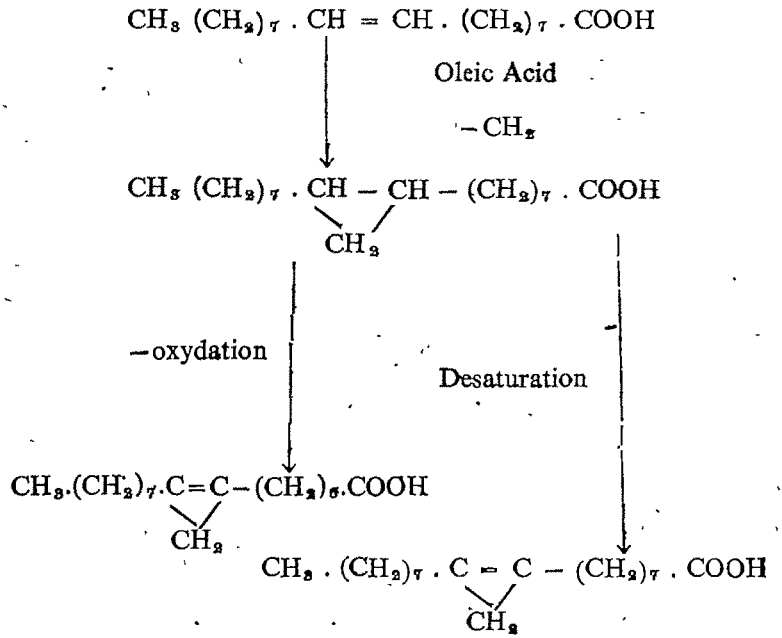


TABLE XVI

$$CH_3CO.SCoA + 7H_2C.CH_2.CO.SCoA + 14NADPH + 14H^+ \longrightarrow CH_3.(CH_2)_{14}.CO.SCoA + 14NADP^+ + 7CO_2 + 7CoASH + 7H_2O$$

TABLE XVII

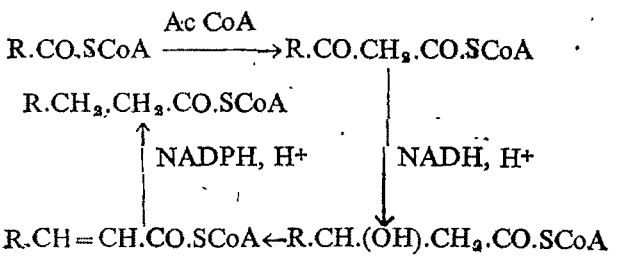
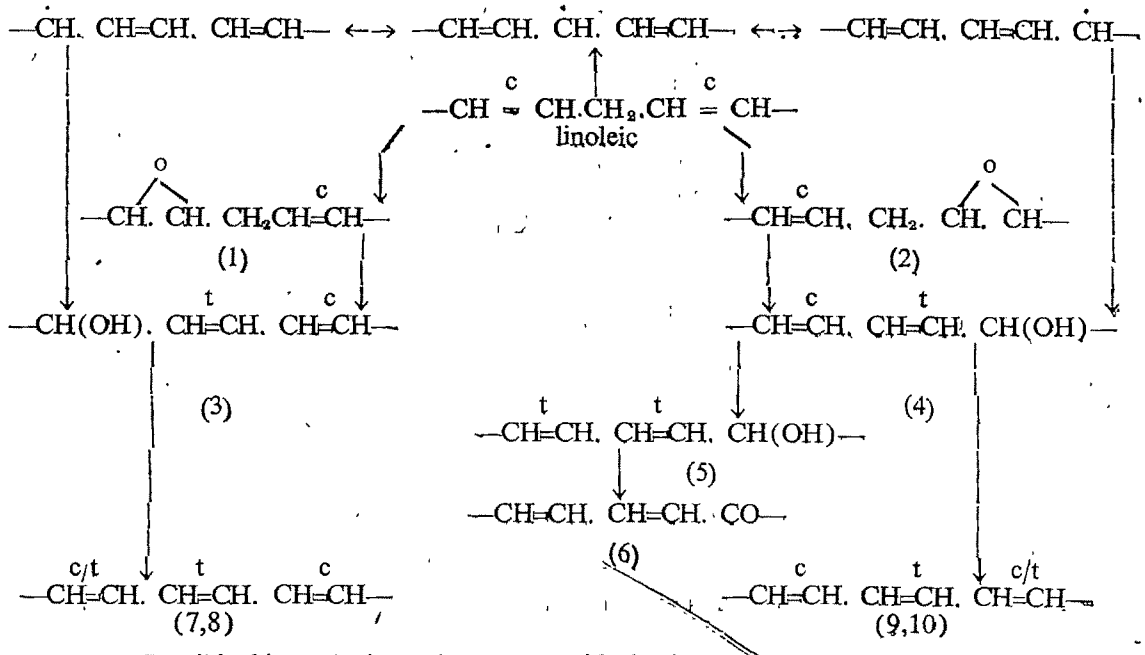


TABLE XVIII



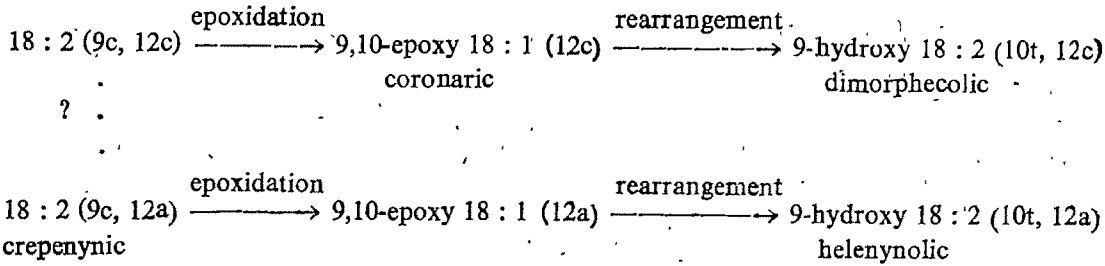
Possible biosynthetic pathways to acids having conjugated unsaturation starting from linoleic acid.

TABLE XVIII (Contd.)

These partial structures represent the following acids : (1) vernolic, (2) coronaric, (3) α- artemesic, (4) and (5) α- and β-dimorphecolic, (6) 9—oxo—octadeca—10t, 12t—dienoic, (7) eleostearic (9c, 11t, 13t), (8) punicic (9c, 11t, 13c), (9) calendic (8t, 10t, 12c), and (10) octadeca— 8c, 10t, 12c—trienoic.

Catalpic acid (18 : 3 ; 9t, 11t, 13c) could result from dehydration of β - artemesic acid or from a similar sequence of reactions starting with linelaidic acid (18 : 2 ; 9t, 12t), which co-exists with the triene acid, or with 18 : 2 (9t, 12c).

TABLE XIX



Possible biosynthetic pathway to α-dimorphecolic and helenynolic acid by epoxidation and rearrangement.

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Part II

PLANT GENETICISTS' CONTRIBUTION TOWARDS THE DEVELOPMENT OF NEW TYPE OF NATURAL FATS

Nature has been always very generous in producing multitude of oilseeds having diverse nature of fatty acids, triglycerides and unsaponifiable flavour components with pigments, antioxidants, vitamines etc. Depending on the fatty acid profile, glyceride structure and the composition of the non-triglycerides the various oils and fats have distinct edible and industrial applications.

However, it should be stated that certain oils having use in frying medium in some countries are considered unfit for consumption in many countries due to certain biological implications.

Mention may be made of erucic acid-rich rapeseed oil containing allyl isothiocyanates as well. The biochemical investigations claim that high erucic acid-rich oils have adverse effects, when fed in a large proportion as a diet to animals and cause cardiolipodosis and later myocardial changes occur. This study has shown that the level of erucic acid above 6 per cent to dietary calories promote definite lipidosis in the rat myocardium.

Soyabean oil, because it contains 7-10 per cent linolenic acid, has a problem of storage as this acid is believed to be involved due to a flavour problem. Safflower oil, owing to its having very high linoleic acid content, is good for people with cholesterol problem. However, such high linoleic acid rich oil in a frying medium does not appear to be very stable and is likely to be involved in the formation of lipid molecules that may be highly mutagenic. Cotton seed oil contains gossypol that is responsible for dark colour content of cotton seed oil and toxicity when used in animal nutrition. This gossypol remains in the meal also after extraction of oil.

All the above biochemical problems associated with the lipid and non-lipids of some specific oils have led to very exten-

sive research programs in developing the concerned oil seeds with complete elimination or with much reduced problem.

The Plant Geneticists have contributed enormously in these areas in developing new variety of oilseeds having altogether different nature of oil and non-oil constituents. The entire approach is based on the manipulation of the genes or the genetic innovation.

The Plant Geneticists have developed practically zero erucic acid variety of rapeseed oil and also very low content of flavour component, the allyl isothiocyanates. Table I (Slide I) shows the fatty acid profile of high erucic acid and low erucic acid rapeseed oils[1].

By genetic manipulation, it has been also possible to produce very high erucic acid rich rapeseed oil for industrial utilisation of erucic acid. It is also interesting to mention here that Downey et al.[2] have reported very recently that by biotechnological manipulation rapeseed oil having high linoleic content about 30 per cent and with much reduced linolenic acid about 3 per cent can be developed so that rapeseed oil can be very close to soyabean oil in respect to fatty acid composition. It is reported by the same authors that rapeseed appears to be more responsive to the biotechnological manipulation with instant inbred plant being produced routinely from the cultivating of anthers and microspores, while fusing of protoplasts to combine desirable cytoplasmic traits from one parent with the nuclear or cytoplasmic characteristics of entirely new oil seeds having new natural oil composition.

Knowles[3] has been able to produce safflower oil having high oleic acid by genetic manipulation. He has been able to identify the major genetic *locus ol* which governs the proportion of oleic and linoleic acid with the genotype *olol* having 72-80 per cent oleic acid in safflower seed oil and the genotype *Olol* 72-80 per cent linoleic acid. The fatty acid profile of some introduction and selection of safflower seed oil are shown in Table II (Slide II). It may be noted from the slide that two varieties of safflower seed could be produced one having high linoleic acid and another having high oleic acid.

It has been possible to modify sunflower oil by appropriate breeding method. Considerable changes in the gross chemical

composition of the seed has been effected. Russian workers were able to increase the oil content of the seed from 35 to 40 per cent thus increasing the average yield of edible product per acre by 30 per cent or more by taking advantage of controlled hybrids and by breeding for control of pests. With controlled environment the following range of oil composition can be produced, namely saturated fatty acids 8 to 40 per cent, oleic acid 12 to 66 per cent and linoleic acid 25 to 80 per cent. The fatty acid composition of different varieties of sunflower seed[4] can be seen from Table III (Slide III) that linoleic acid can be varied from about 38 per cent to as high as to about 74 per cent with corresponding oleic acid from about 15 per cent to as high as 51 per cent.

Soyabean oil has been also studied quite extensively to alter its fatty acid profile by genetic manipulation in soyabean oil seed crop. The primary objective of genetic manipulation is to produce soyabean oil with much lower concentration of linolenic acid that is implicated with off flavour of the oil.

A very recent report[5] indicates that germplasms for the desired trait for zero or low linolenic can be available. A selection (N 84—6002) from the progeny of germplasm contains 3.5 $\pm$ 0.50 per cent linolenic acid, when grown in several environments. There is also increasing protein content in the seed.

A variation in fatty acid profile in different genetically bred soyaseed oil is shown in Table IV (Slide IV).

Cotton seed oil is characterised by a phenolic component gossypol which is well known to be responsible for the troublesome dark colour of cotton seed oil; it causes discoloration of the egg when used as food and also creates metabolic disturbance when fed to non-ruminant animals in excessive amounts. Plant geneticists have also succeeded in developing glandless cotton seed oil which is essentially free from gossypol (Fig. I Slide V).

A lot of genetic research has been in progress to produce cotton seed with low gossypol content or free of gossypol. Gossypol is contained in pigment glands present in the cotton-seed kernel. Glandless cottonseeds are essentially free from gossypol and produce very light colour crude oil or meal.

A number of cotton seed variety has also been developed in India[6] whose composition is shown in Table V (Slide VI).

The elimination of gossypol from oilseed and the variation of fatty acid composition in oil have led to the utilisation of cottonseed oil as food fat and also food application of defatted and protein rich isolate which is now being used in human nutrition.

In recent years successful results have been obtained by using biotechnological methods such as tissue culture. Dr. A. T. James of Unilever Research has used plant cell cultures for plant multiplication of selected premium oil-yieding plants for improving agricultural production. While the cost per plant rules out the annual crops such as rape and soyabean, perennial crops such as cocoanut and oil palm are amenable. Both crops are propagated by seeds and since the seed is derived from parents not inbred to stable but the progeny are highly variable. James has been successful in developing selected oil palm by this method. Tissue from the chosen plant (that are already growing in a plantation with consistent higher yield, response to fertilisers etc.) is disinfected and put into a defined growth medium. The tissue respond by producing cells known as callus Fig. 2, 3 (Slides 7, 8) that grow moderately rapidly and can be mutiplied up to any desired scale by detaching pieces and subculturing. By appropriate change in the culture medium, these cells can be encouraged to produce compact cell masses (called embryoid) that closely resemble the normal embryo from the seeds. The embryoids will also multiply indefinitely under the correct conditions and can then be induced to form roots and shoots and hence small viable plants. Each of these plants is genetically identical to the parent plant from which the original piece of tissue was obtained (a clone). It was initially possible to increase the yield of palm oil from 6 tonnes/ha to 8 tonnes/ha in Malaysian palm plantations. The present indications are that the yield of up to 10 tonnes/ha is possible. Two unexpected additional advantages have emerged (a) a considerable variation in fatty acid composition between clones and (b) a considerable variation in carotenoid contents between clones. It has been possible to produce, apart from those giving the conventional palm oil, others that give a higher oleic level, or a higher

palmitic level, or a higher linoleic level. This gives the growers the possibility of more specialised oils as well as conventional palm oils. The higher cost of tissue cultures compared with conventional seeds is soon recovered in 2 years so that in the next 20 years the growers reap a major benefit. The same approach is being taken with cocoanut palm and one would hope the similar advantages will accrue. A new dimension has been added by the ability of genetic engineering to change the proteins, crabohydrate and composition of major oilseeds towards the composition required by the end users. All these would result ultimately in requiring less processing for human use and better seed proteins for both the animal feed and human food industries.

The recent developments in biotechnological area have raised hopes for producing oils as supplement to existing supply of plant seed oils. The first is production of oils and fats with the help of microorganisms and second is production of oils from microalgae. During the two World wars although the production of microbial oils in Germany was without apparent success, it could be established that certain microorganism could be manipulated into producing considerable amount of extractable and acceptable oils. The work carried out in those early years established the main principles upon which lipid accumulation could be encouraged in microorganisms. The conditions which had to be satisfied was that the organsim had to be grown so that its multiplication was eventually curtailed by the exhaustion of a nutrient other than carbon from the medium. The organism then converted the excess carbohydrate substrate into lipid without further cell proliferation. Cells therefore, 'fattened'. This led to the now-classic two stage profile for lipid accumulation in batch culture becoming established. The vast majority of oleogenous microorganisms are to be found in yeasts and molds. Tables VI and VII (Slides IX, X) give the list of some organisms which may become the most prolific and efficient means of producing oils outside the conventional agriculture. Some of these accumulates more than 25 per cent of their bio-masses as lipids. The nature of lipids in both yeasts and molds are predominantly glycerides. Table VIII (Slide XI) gives the analysis of major lipid classes and Table IX (Slide XII) fatty acyl

groups of total lipids from some yeasts and molds. It is interesting to note that (1) the distribution of fatty acids in glycerides itself follows that found in plant i.e. the 2-position is occupied almost exclusively by unsaturated fatty acids. Also the fatty acids are mainly C_{16} and C_{18} in chain length and analysis of compositions show many similarities to plant seed oils. The predominant fatty acids in order of usual abundance are oleic acid; palmitic acid, linoleic acid, stearic acid and palmitoleic acid. Some genera will produce acids in a different order of abundance and some may produce relatively large proportion of total fatty acids as γ-linolenic acid. Unusual fatty acids are not very common although branched chain and hydroxy acids are found in some. Thus it is seen, yeasts and molds can produce a variety of different oils many of which could be used, if necessary, as substitutes for existing commercial plant seed oils. It is now possible to build production fermentation plants which are capable of producing 100,000 tonnes of biomass/annum at a cost which may be competitive with plant fodder material such as soyabean meal. The process of microbial oil production single cell oil (SCO)—is fundamentally no different from that of single cell protein (SCP) production.

The exceptionally large photosynthetic production of microalgae (phytoplankton) received much attention over the years. Oil production by microalgae is a relatively new application. It is stated that compared to areal yield from soyabean, microalgae could produce up to 30 tonnes more oil. Moreover, the algal crops offer many possibilities for further enhancing oil production because cell generation time is short and physiological manipulation can be substantiated. It has been estimated in the case of microalgae that a 3.3 to 5.6 per cent photosynthetically available light conversion efficiency is achievable. It is much higher than terrestrial plants with the exception of sugarcane. The lipid content of algae can vary from 15 to 50 per cent of dry weight (Fig. 4, Slide XIII). In addition to the exceptional oil productivity the photosynthetic ability of microalgae (being single cell organisms) allows them to grow on CO_2 and mineral nutrients without an expensive organic carbon source. Manipulation of the cultural environment or genetic make-up of the culture is easy. Either of these manipulations could result

in enhanced oil productivity or a tailoring of the lipid mix to provide a highly desirable product (Table X, Slide XIV).

The growth period and environmental conditions of phytoplankton lipid study has been reviewed recently. In general it has been seen that the fatty acids in these methods contain 12 to 22 carbon and the major fatty acids in most species contain C_{16} and C_{18} saturated and unsaturated groups similar to vegetable oils. Although at this point of time R and D efforts are being expanded, field demonstrations are needed for oil yield data under realistic conditions. Based on very conservative figures for capital and operating costs, microalgal oil might be produced within a range of $ 0.25 to 2.0 per gallon depending on the nutrient source which at this point of time is higher than the market prices. However, it is predicted that the potential for further improvement in processing technology, yields and quality is so great that microalgae must be considered as an excellent source for future vegetable oil production.

It would thus appear that apart from genetic manipulations and tissue culture methods, vegetable fats of the usual type are also obtainable from microbial sources and microalgael sources, thus expanding the horizon of source materials beyond conventional agricultural methods without the use of land whose avaailability is limited in our country.

Projected World Biotechnology Product Sales (1988-1990)[5]

Product	Billion US
Energy	9.4
Agriculture	5.7
Food	3.7
Drugs	2.9
Plastics	2.6
Chemicals	2.5
Others	0.3
TOTAL :	27.1

5

TABLE I Fatty acid compositions of High Erucic and Low Erucic rapeseed oil[1]

	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	$C_{22:0}$	$C_{22:1}$	$C_{24:0}$
Low Erucic Acid	0.2	2.5	0.6	0 9	50	18	6	0.1	0.1	0.5	5.0	0.2
		6.0		2.1	66	30	14	1.2	4.3			
High Erucic Acid		2	—	2	34	17	7	—	9	—	26	—

TABLE II Fatty acid compostiton of some introductions and selections of Safflower[3]

UC designation or variety name	Fatty acid composition			
	Palmitic	Stearic	Oleic	Linoleic
India 65-204	6.5	2.7	31.6	59.2
India 65-246	6.7	1.9	24.6	66.8
India 65-465	6.4	1.7	25.3	66.6
Australian introduction	7.6	1.0	28.3	63.1
US-10 selection	7.5	2.1	25.8	64.6
US-10, high linoleic	7.9	2.4	17.9	71.8
UC-1, high oleic	5.9	1.6	78.8	13.7

TABLE III Fatty acid distributions in Sunflower oils from various sources and varieties[4]

Fatty Acid	Weight Per cent								
	Russian variety		American variety				Canadian variety		
	a	b	c	d	e	f	g	h	i
14 : 0	0.2	0.1	0.1	0.1	—	—	—	0.1	0.2
16 : 0	6.0	6.2	5.5	6.1	5.9	5.2	6.1	6.2	7.0
16 : 1	0.1	0.1	0.1	0.1	—	—	—	0.2	0.1
17 : 0	tr	tr	tr	tr	—	—	—	0.1	—
17 : 1	—	—	tr	—	—	—	—	tr	—
18 : 0	5.6	4.4	4.7	4.2	4.7	4.4	3.7	4.6	4.0
18 : 1	17.8	17.0	19.5	14.9	26.4	50.9	16.4	26.4	21.5
18 : 2	68.7	71.1	68.6	73.5	61.5	37.9	73.7	61.0	65.9
18 : 3	0.2	0.1	0.1	0.2	—	—	—	0.1	0.2
20 : 0	0.3	0.2	0.3	0.2	0.5	0.5	—	0.5	0.6
20 : 1	0.1	tr	0.1	—	—	—	—	—	—
22 : 0	0.8	0.8	0.9	0.7	0.7	0.7	—	0.5	tr
22 : 1	—	—	—	—	—	—	—	0.3	0.5
24 : 0	0.1	tr	0.2	—	—	—	—	—	—

a Armavirec, b — 8931 VNIMK, c — Mingreen, d — Sample from Minnesota Linseed Oil Co. of 1968 production, e — Variety grown in Minnesota, f — Variety grown in Texas, g — Russian variety 'Peredovnik', grown in Canada mean value of oil from 1964 seed from 10 locations

TABLE IV Fatty acid distribution for some commercial and experimental soyabean oils reported during the period[a] 1965-1976 (166, 475, 478, 479)

Fatty acid	Weight per cent					
	Commercial oil		Experimental oil			
	(478)	(166)	(479)[a]	(479)[b]	(475)[c]	(475)[d]
12 : 0	tr	—	—	—	—	—
14 : 0	0.1	—	—	—	—	—
15 : 0	tr	—	—	—	—	—
16 : 0	0.5	12.0	—	—	—	—
16 : 1	tr	0.5	—	—	—	—
17 : 0	tr	—	—	—	—	—
18 : 0	3.2	3.6	—	—	—	—
18 : 1	22.3	23.7	42.0	32.8	37.5	36.9
18 : 2	54.5	51.4	38.3	46.4	44.2	48.5
18 : 3	8.3	8.8	4.8	7.0	4.0	5.5
20 : 0	0.2	—	—	—	—	—
20 : 1	0.9	—	—	—	—	—

a. Genotype selection F_4 from Pl 90, 406 and Pl 92, 567
b. Parent Pl 90, 406 genotype
c. Grown at Tifton Ga average daily summer temperature 78°F, 16.9 in of rain 1973, Group A.
d. Grown at Blairsville, Ga., average daily summer temperature 64.5°F, 27.2 in of rain, 1973, Group A

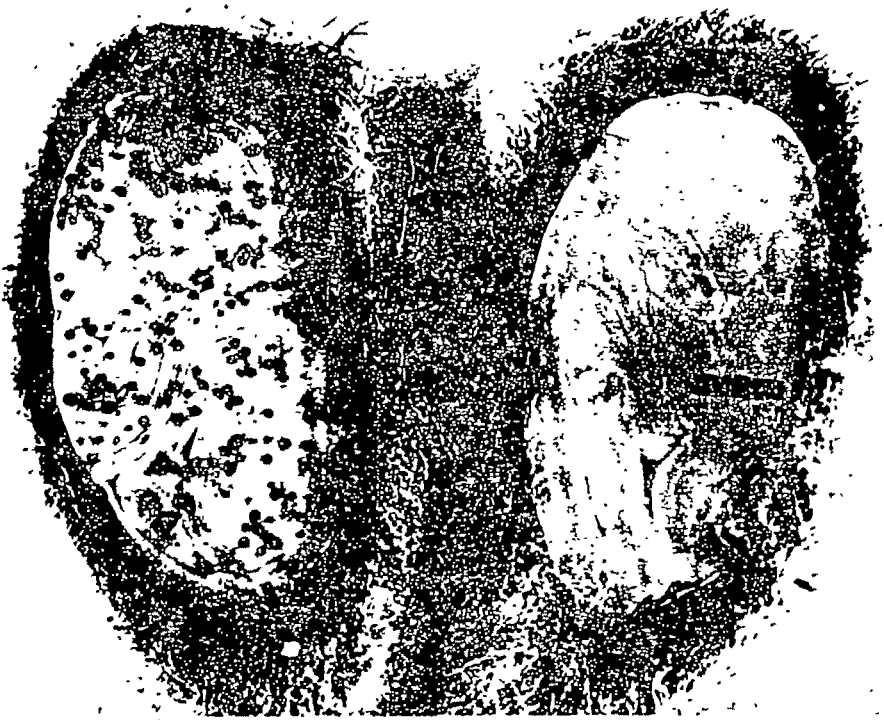

Fig. 1.

Longitudinally sectioned cottonseed : glanded seed on the left (dark spots in the kernel are glands) and glandless seed on the right.

TABLE V Fatty acid compositions of cottonseed oil varieties in India[d]

Sl. No.	Variety	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{20:0}$	$C_{22:0}$	$C_{18:1}$	$C_{18:2}$	C.P.A.
1.	Giza-7	0.7	29.9	2.5	0.3	0.5	17.7.	47.6	0.8
2.	Varalaxmi	1.2	28.9	2.8	0.3	0.4	15.0	50.5	0.9
3.	CBS 156	1.9	23.2	5.2	0.3	0.4	20 9	47.2	0.9
4.	Hybrid-5	2.2	26.7	3.2	0.3	0.4	15.9	50.2	1.1
5.	Laxmi	1.2	26.7	2.9	0.3	0.4	17.4	50.3	0.9
6.	NHY-12	1.2	25.5	3.4	0.3	0.4	18.0	50.5	0.8
7.	Hybrid-4	0.3	27.1	3.4	0.3	0.4	14.1	53.8	0.6

C.P.A = Cyclopropenoid acids

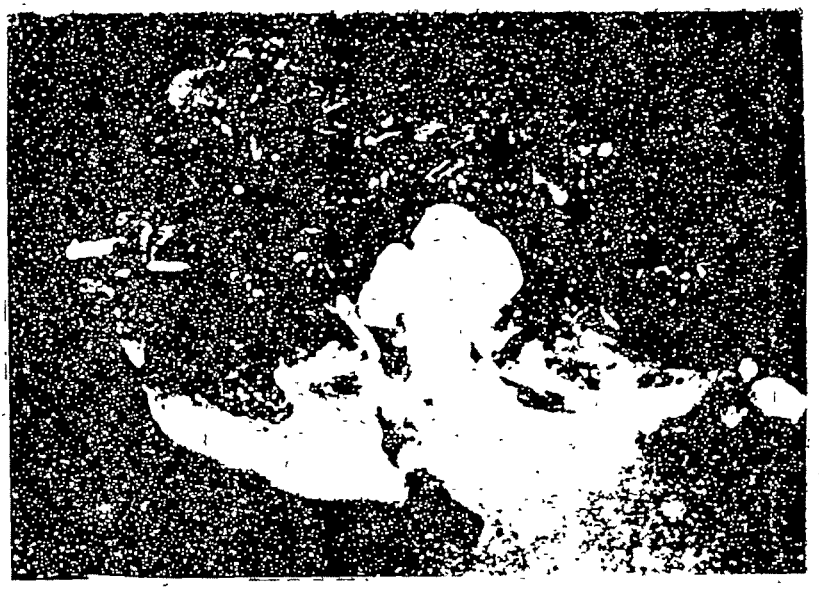

Fig 2.
Embryoids on primary callus.

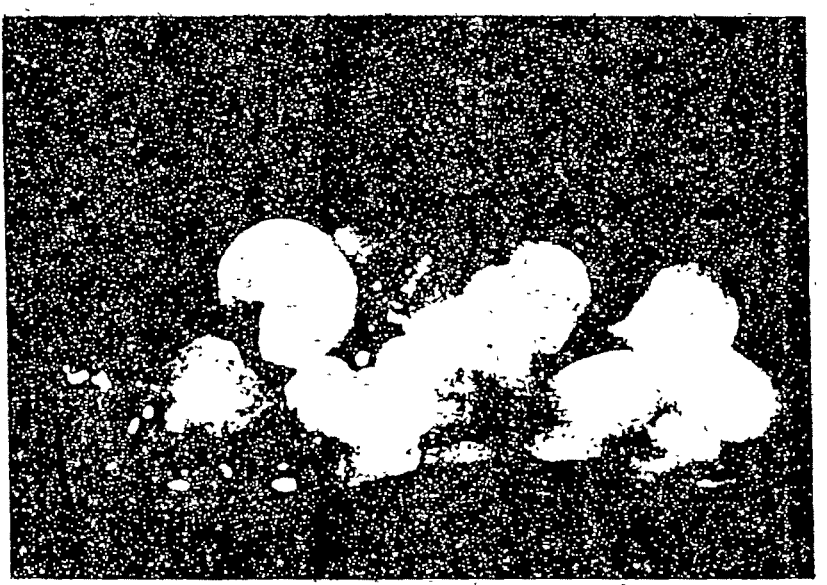

Fig. 3.
Close up of cluster of embryoids.

TABLE VI Oleaginous Yeasts and Their Lipid Content[7]

Species	Lipids Content %	Species	Lipid Content %
Candida curvata	58	Lipomyces starkeyi	63
Candida lipolytica	36	Lipomyces tetrasporus	64
Candida paralipolytica	32	Rhodosporidium toruloides	66
Candida sp. 107	42	Rhodotorula glutinis	71
Cryptococcus terricolus	65	Rhodotorula graminis	41
Endomycopsis vernalis	65	Rhodotorula mucilaginosa	28
Hansenula saturnus	28	Trichosporon cutaneum	45
Lipomyces lipofer	63	Trichosporon pullulans	65
(= lipferus)		Trigonospis variablis	40

TABLE VII Oleaginous Fungi and their Lipid Content

Family	Species	Lipid Contents %
Entomophthorales	Entomophthora coronata	45
	Cunninghamella echinulata	45
	Cunninghamella elegans	56
Mucorales	Mortierella vinacea	66
	Mucor albo-ater,	45
	circincelloides	65
Peronosporales	Pythium ultimum	49
	Aspergillus fischeri	53
Ascomycetes	terreus	57
	Chaetonium globosum	54
	Fusarium bulbigenum	50
Basidomycetes	Ustilago zeae	51

Dermatophyte fungi are known which accumulate up to 44% lipid but these should be regarded as pathogenic fungi and therefore not suitable for commercial exploitation.

TABLE VIII Lipid Analysis of Some Oleaginous Yeasts and Molds[7]

	Relative % of major lipid classes*						
	TAG	DAG	MAG	FFA	FS	ES	PL
Yeasts							
Candida 107	72-83	——	11-21	——		1-2	5
Cryptococcus terricolus	91.6	2.5	0.3	2.6	0.6	0.4	1.8
Lipomyces starkeyi	88	5	—	5.0	—	1)	2
Rhodotorula glutinis	84.4	—	—	10.0	0.4	3.0	2.2
Molds							
Fusarium oxysporum	48 3	3.2	1.0	8.3	10.4	1.3	27.5
Penicllium lilacinum	——	92.3	——	3.4	—— 4.3	——	
Phythium irregulare	70 8	5.5	0.7	8.4	—	1.5	13.5
Tricholoma nudum	92	—	—	—	2	1	5
Ustilago zeae	16	—	—	4.0	10	21	12

* TAG = triacylglycerols ; DAG = diacylglycerols ; MAG = monoacylgycerols ; FFA = free fatty acids ; FS = free sterols ; ES = sterol esters ; PL = phospholipids.

TABLE IX Fatty Acyl Groups of Total Lipid from Various Yeasts and Molds[r]

	Relative % fatty acyl composition							
	12 : 0 or shorter	14 : 0	16 : 6	16 : 1	18 : 0	18 : 1	18 : 2	18 : 3	Others
Yeasts									
Candida curvata D	—	tr	32	—	15	44	8	—	
Cryptococcus terricolus	—	—	18	1	6	60	12	2	24 : 0, 1%
Lipomyces lipofer	—	1	16	3	3	62	9	1	
Trichosporon cutaneum	—	tr	30	—	13	46	11	—	
Molds									
Aspergillus nidulans	—	1	21	1	16	40	17	tr	20 : 0, 1%
Entomorphthora obscura	41	8	37	tr	7	4	tr	tr[a]	20 : 5, tr
Penicillium lilacinum	—	tr	16	3	2	40	13	—	
Phythium irregulare	1	6	26	15	5	26	5	6[a]	20 : 0, 7%
Thicholoma nudum	—	tr	29	1	8	32	29	tr	22 : 0, 1%

a All cis 6, 9, 12-18 : 3 (γ-linolenic acid); in all other cases it is the alt cis 9, 12, 15-18 : 3 isomer (α-linolenic acid).
tr = trace

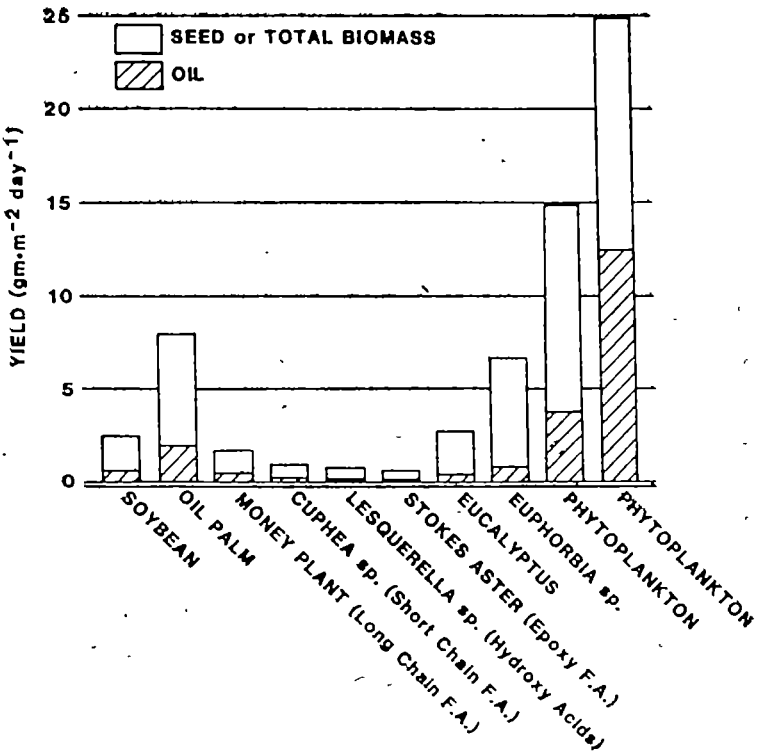
25
20
15
10
5
0
SEED or TOTAL BIOMASS
OIL
YIELD (gm.m⁻² day⁻¹)
SOYBEAN
OIL PALM
MONEY PLANT (Long Chain F.A.)
CUPHEA sp. (Short Chain F.A.)
LESQUERELLA sp. (Hydroxy Acids)
STOKES ASTER (Epoxy F.A.)
EUCALYPTUS
EUPHORBIA sp.
PHYTOPLANKTON
PHYTOPLANKTON

Fig. 4.

TABLE X Comparison of Agriculture vs. Microalgae Aquaculture[7]

	Agriculture	Aquaculture
Commercial acceptance	Established	R & D
Yields	Low	High
Harvesting		
Technology	Established	Developing
Residue Waste	High	Low
Land Requirements	High quality land	Marginal land
Water		
Quantity	Evapotranspiration	Evaporation
	Evaporation	
	Runoff	
Quality	Freshwater	Freshwater
		Saltwater
Fertilizer	Excess is lost	No excess losses
Genetic optimization	Mostly achieved	As yet untapped

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Part III

THE FUTURE OF OILS AND FATS INDUSTRY IN INDIA IN 2000 A. D.

India has been passing through the phase of acute shortage of vegetable oils for the past several decades. In fact, the country has become a major importer from the status of one of the major exporters of oils and oilseeds in the world.

Even the per capita consumption is still at a very low level about 5 kg/annum. The country imported, for instance, 16 lakh tonnes of vegetable oils for edible purpose in the year ending 1984—1985. The demand pattern indicates that the import has to be increased to about 20 lakh tonnes in the current year. In the year 2000 A. D. the demand pattern will quite significantly increase along with increase in the national income and the per cent of population.

It is estimated that the demand could be about 87. 5 lakh tonnes at 3. 5 per cent growth of national income while the supply position could be 51. 5 lakh tonnes, showing deficit of about 35. 9 lakh tonnes. If the national income is considered at 5 per cent growth rate, the demand will increase further and is projected to be 122. 5 lakh tonnes with a supply situation at the same level (51. 5 lakh tonnes) showing thereby a deficit of 71 lakh tonnes (Table 1/Slide I).

A number of action plans has been taken up to augment the oils and fats supply situation in the country. The first and foremost importance has been given to increase the production of major oilseeds such as ground nut, mustard, soyabean, sunflower by bringing more land under irrigation, using improved varieties of oilseeds like high yielding varieties, and increasing the use of other agricultural inputs, by multiple cropping or suitable intercroping on marginal or dry farming lands (Table 2, Slide 2).

All out efforts are also being made to organise collection of various tree borne oilseeds and various by-product oil sources (Table 3, Slide 3). Planning is also going to cultivate palm plantations with high yielding clonal palm varieties for the

production of palm oil in the country in the areas like Kerala and Andaman & Nicobar Islands.

In addition to the steps undertaken for increasing the production of oilseeds, considerable developments of technology have been in progress throughout the world in order to utilise oils and fats and the non-oil constituents in a much better manner. Even today technologies are being innovated and improvised and also new emerging technologies are being considered for processing oilseeds and their constituents to meet the demand for the year 2000 A. D.

New product mix can be developed and in fact have been receiving much consideration in India to meet the needs of consumers. The new technologies that can be applied to processing the oilseeds and oils and fats include extraction, refining, modification and new products mix, some of which have enormous commercial feasibilities in India.

New Extraction Technology :

In view of the increasing cost of hexane, the biorenewable solvents like ethanol and isopropanol are explored for extraction of oils in place of hexane to save energy cost, to deacidify partially the extracted oils and also simultaneously improve the quality of deoiled meals.

Supercritical carbon dioxide is considered to be an ideal solvent to extract oils and also simultaneously fractionate different components of oils. The details about the biorenewable solvents in extraction are shown in Fig. 1, Tables 4, 5 (Slides 4—6).

Direct extraction of oil from high oil bearing seeds without any preprocessing can be an important technology for adoption in India. Direct extraction which includes the combination of percolation, flaking and immersion method of extraction, has been developed in the name of the Direx process in Italy. Direct extraction of intact whole oilseed kernels with hexane would be worth investigating in the case of particularly edible oilseeds that could give protein-rich and fat-lean kernels suitable for snacks and confectionery.

Refining Technology :

New refining technologies are coming up in order to process high free fatty acid and dark colour oils which are otherwise extremely difficult to refine and bleach by conventional refining and bleaching procedures. Some of the refining methods which need special mention include physical refining and miscella refining with a single solvent or mixed solvent and their combination.

Physical refining, which was found to be extremely successful in refining, bleaching and deodorising palm oil, has been simultaneously applied for a number of other oils specially having high f.f.a. The process involves degumming, prebleaching and steam stripping at high temperature of 240-260°C and at low pressure of 4-6 mm. This physical refining process should be adopted in India for refining and deodorisation of a number of edible oils (Table 6 and Slide 7).

Refining of high f.f.a. oils in miscella phase with or without another polar solvent such as alcohol/isopropanol is a commercial proposition for high f.f.a. oils like rice bran oil and a host of other oils. The miscella refining process consist in mixing with caustic soda solution at ambient temperature or at a temperature between 50-60°C followed by removal of soap stock in a specially designed centrifuge or in a series of decanters.

The miscella phase and soap phase after further washing can be further recovered for further treatment. In the case of refined miscella, the bleaching treatment can be carried out also in the miscella phase with a better colour removal and with very little oils loss after filtration. The entire refining and bleaching process, however, need a special design, fire proof and leak proof equipment. The typical results of refining and bleaching of rice bran oil (RBO) as obtained by the miscella refining process are shown in Tables 7, 8 (Slides 8—9).

A commercial plant is now in operation in India to deacidify the high f.f.a. rice bran oil by mixed solvent refining process.

Modification of oils and fats by hydrogenation reaction:

Oils and fats are modified by traditional hydrogenation pro-

cess to make vanaspati, bakery fat, margarine fat base etc. The traditional nickel catalyst still is used in India but it may be mentioned that a number of new kinds of nickel catalysts such as Sulphur promoted catalysts have been commercially produced elsewhere which have enormous potential in improving selectivity and thermal properties for specific products preparation[3].

Such new catalysts need to be tried in Indian hydrogenation industry Fig. 2 (Slide 10) particularly for margarine manufacture which is being developed in India.

Modification of fats by interesterification reaction :

In view of the cost of hydrogenation, the interesterification process has been gaining importance as an alternative process of modification of oils and fats to make new kinds of vanaspati, shortening and margarine bases.

The process utilises the fatty acid interchange in various triglyceride molecules of fats in random or in directed manner in presence of a simple base catalyst like NaOEt, and NaOMe, Na-K alloy, NaOH-glycerol etc. Some of the interesting results are given in Tables 9—10 (Slides 11—12).

This process is well known outside India but the process has not received Government approval as yet in India.

The interesterification process as such and in combination with hydrogenation has tremendous potential in producing edible fat product of desirable plastic range and consistency from a variety of indigenous and imported oils and fats to cater to the specific needs of confectionery, bakery and other industries.

Modification of fats by blending :

Fats can be modified for specific uses by blending sample e.g. highly hydrogenated fat or high melting interesterified fat can be blended with a liquid oil to make edible fat products. Blending of some stearin fractions can be gainfully utilised in making confectionery fat products (Table 11, Slide—13). Blending can also be very useful in making cheaper cooking oil and also in making stable frying oils. In fact blending of oils and fats has recently been permitted for making available cooking oil at a cheaper price to the consumers.

Modification of fat by lipofrac fractionation :

Fats are modified by fractionation into useful products. The fractionation can be achieved by dry method by classical technology of panning and pressing, from a suitable solvent at low temperature, and by using detergent at low temperature. Commercially the detergent fractionation process has assumed in recent years tremendous importance. A very successful application of this process has been made for palm oil to produce about 20 per cent stearin and 80 per cent olein (Fig. 3, Slide—14). The stearin is known as palm stearin and has much scope of utilisation in making edible fat products, soaps and oleochemicals. The olein known as palm olein in commerce is a stable frying oil. It has also wide scope of utilisation in making edible fat products by hydrogenation and interesterification. The detergent fractionation process is quite well known outside India and is adopted commercially in many countris for the purpose of fractionation and also the removal of waxes from vegetable oils. This fractionation process could be considered for adoption in India for which Government approval will be needed. This process can be adopted in India for dewaxing of rice bran oil (Table 12, Slide—15) and for segregating a number of vegetable oils and indigenously grown palm oil.

Modification of fat by Biotechnology :

Biotechnology is regarded as emerging technology in the field of oils and fats industry. There are a number of biochemical transformation possible for fats and oils. Among these, mention be made of enzymatic interesterification and enzymatic hydrolysis. A great deal of work has been done on these areas that indicate that biotechnology is the futuristic technology in making very specific and edible fat products and also for industrial application. Some of the applications are given in the Tables 13—16 (Slides 16—19).

In India biotechnology has a great deal of scope that can be developed in the utilisation of minor oils of India.

In addition to the adoption of newer technologies it is essential that we think of new product mix like margarine and

low cost spread fat, confectionery fats, bakery products utilising oilseed protein, flavoured oil etc. Margarine can be used as substitution of butter fat as a spread fat and also other varieties of margarines for cooking medium and bakery products should be produced in India. Margarine is an established industry outside India. Its production has yet to be in the regular industrial scale in India. It is, however, admitted without any reservation that the vast majority of the population of the country cannot afford the use of butter as spread fat. The only alternative of the butter fat is margarine which can be produced from our various indigeneous and imported oils by adopting known technology (Table 17, Slide 20). It is unfortunate that though colour and flavour in margarine are permitted outside India, the Government of India have not yet permitted the use of synthetic colour and flavour. Margarine will provide the nutritional benefit to the people by providing poly unsaturated fatty acid (PUFA) and vitamins. Margarine normally contains 16 per cent moisture but products having lower fat content with spreadability can be made for low cost spread fat products for the consumers.

We can also consider producing cooking fats in the form of margarine instead of the conventional frying fat which can be accomplished to a large extent by providing proteins to the aqueous phase of margarine.

We should also think of extending frying oils thereby saving consumption of oils by taking advantages of suitable emulsifiers that foam the frying oil during frying. It is due to the foaming effect that the high demand of heat transfer medium is created thus substantially reducing the level of oil consumption in frying of food items.

Bakery industries are not yet developed to the extent desirable. The primary bakery products in India are bread and biscuits and use 70,000 tonnes of oils and fats. Oils and oilseed proteins can find use in bakery products as an efficient and acceptable vehicle to spread consumption. One of the major application of oils and fats in bakery products particularly in bread industry can be used as bread improvers.

In bakery products specially plastic fats prepared by using interesterification process can be enormously useful.

Another area can be the utilization of various oilseed protein in enriching the flours used for baking in order to manufacture protein fortified bread, biscuits and other snacks, foods etc.

In order to utilize various oilseed protein, it is highly important that groundnuts, rapeseed, sunflower meals etc. are properly detoxified for the above usages for which more technical and R and D work should be done.

The technologists so far have dealt with the edible products where most of the new technologies will be tried and developed for use in 2000 A.D. and also new technologies can be envisaged and can come up to have an impact on oil technology by the year 2000 A.D. It can also be stated that in the field of non-edible industries we should envisage and develop newer technologies for production of oleochemicals and also newer types of oleochemicals to cater to the various chemical industries.

TABLE I Supply and Demand Projections till 2000 A.D.

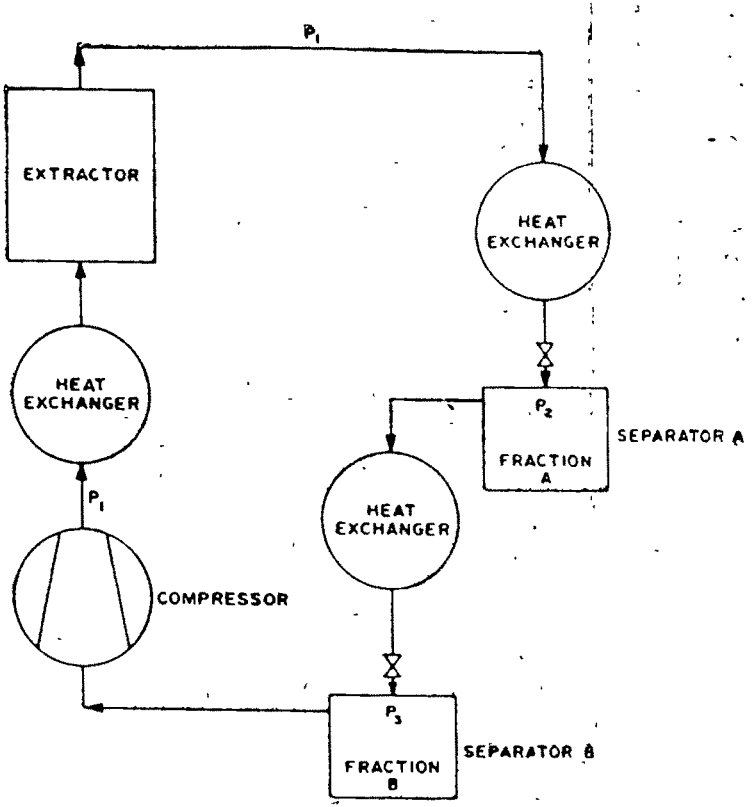
Year	Supply	Demand (lakh tonnes)		Demand (lakh tonnes)	
	Lakh tonnes	at 3.5% growth in national income		at 5% growth in national income	
1985—86	35.58	44.43	(— 8.85)	47.00	(—11.42)
1990—91	40.26	55.69	(—15.43)	64.70	(—24.44)
1995—96	45.55	69.80	(—24.25)	89.06	(—43.51)
2000—01	51.54	87.47	(—35.93)	122.59	(—71.05)

TABLE II Future Strategy in Oilseed Development

Oil	Phase I (lakh tonnes)	Phase II
Soyabean	3	—
Groundnut	1	10
Rape / Mustard	1	2
Sunflower	4	—
Rice bran	3	3
Oilcake	2.5	1
Organised sector cultivation	—	3
Barter	2	1
	16.5	20

TABLE III Oil Bearing Materials for Exploitation

Oil	Potential (in lakh tonnes)
Rice bran	6
Sal	6
Mowrah	1.7
Water melon	0.06
Maize germs	0.20
Jute seed	0.08
Wild Castor	0.04



Simplified flow diagram for the liquified or supercritical gas extraction process.

FIG. 1

TABLE IV Advantages and Disadvantages of Liquified and Supercritical Gas Processing

Advantages	Disadvantages
Liquids may be selectively fractionated during extraction or recovery to produce high quality oils with low free fatty acids.	High operating pressure of 1,000 to 10,000 psi.
Solvent easily removed from oil and meal products by evaporation	Batch processing results in low capacities
Inexpensive raw material obtained as by-product from alcohol fermentation	
Extracted cotton seed meals are light coloured ; low working temperatures results in little protein denaturization	
Non flammable, nonexplosive, nontoxic and does not contribute to environmental pollution	
Can be energy saving	

TABLE V Advantages and Disadvantages of Alcohol Processing.

Advantages	Disadvantages
25 percent reduced energy consumption by the use of recirculation techniques which minimize distillation requirements	May have to purify miscellas from phosphatides carbohydrates and other monoglyceride extractives
Can remove antinutritional factors such as gossypol, aflatoxins and chlorogenic acid	Repeated recirculation of solvent could impart undesirable odors to protein products
Can produce high quality oil with low free fatty acids	Because of polar and hydrogen bonding, alcohols are absorbed strongly on most meals and are difficult to remove
Can produce additional by-products such as lecithin, sterols and saponins	In some cases, oil bearing materials must be dried prior to extraction to minimize dilution of solvent
Greater industrial safety, lower toxicity	Lower solvent power

TABLE VI Deacidification of degummed, dewaxed and pre-bleached rice bran oil by high *vacuum steam stripping*[1]

Free fatty acid (%) in crude rice bran oil	f. f. a (%) in degummed and dewaxed oil	Stripping condition			f. f. a. (%) in the stripped oil
		Temperature °C	Time hr.	Pressure mm. Hg.	
20.5	20.0	220	0.5	3—5	8.9
			1.0		6.4
			2.0		4.7
			3.0		3.8
20.5	20.0	240	0.5	3—5	8.2
			1.0		5.2
			2.0		2.7
			3.0		2.1
20.5	20.0	260	0.5	3—5	7.0
			1.0		4.7
			2.0		2.0
			3.0		1.7
20.5	20.0	280	0.5	3—5	5.6
			1.0		3.7
			2.0		1.4
			3.0		0.8

TABLE VII Purification of degummed[a] and dewaxed[b] rice bran oil by miscella refining in hexane and bleaching of desolventised oil[1]

Free fatty acid (%) in crude rice bran oil	Oil content in the miscella (% w/v)	Refined and bleached oil Characteristics			Refining factor	Unsap Matter (%)
		f. f. a % in the oil	Lovibond colour (1 cm. cell)			
			Y	R		
15.3	60	0.20	4.0	0.7	1.4	1.6
20.5	60	0.18	3.0	0.6	1.4	1.6
30.2	60	0.22	5.7	0.7	1.6	1.7

a degummed by 85% phosphoric acid (1 kg/tonne)

b miscella dewaxing at 15° ± 1°C for 6 hrs.

TABLE VIII Characteristics of Refined and Bleached Rice Bran Oil[a]

Conditions : Oil content in hexane = 60% w/w Temperature during neutralisation = 30°C (ambient)

Mixing time = 30 min. Setting time = 2 hr.

	Mixed solvent alkali refining						
% FFA in Crude oil	Alcohol	% excess alkali used	% FFA in the refined oil	Lovibond colour (1 cm. cell) Y	R	Refining factor	Unsap. matter
20.5	Ethanol	20	0.20	2.3	0.2	1.3	1.8
		50	0.16	1.2	0.2	1.4	1.6
	Isopropanol	20	0.26	4.2	0.4	1.5	2.2
		50	—	—	—	—	—
30.2	Ethanol	20	0.28	7.2	1.1	1.4	2.0
		50	0.22	6.8	0.9	1.4	2.0
	Isopropanol	20	0.33	8.2	1.2	1.5	2.4
		50	0.27	7.0	1.1	1.6	2.0

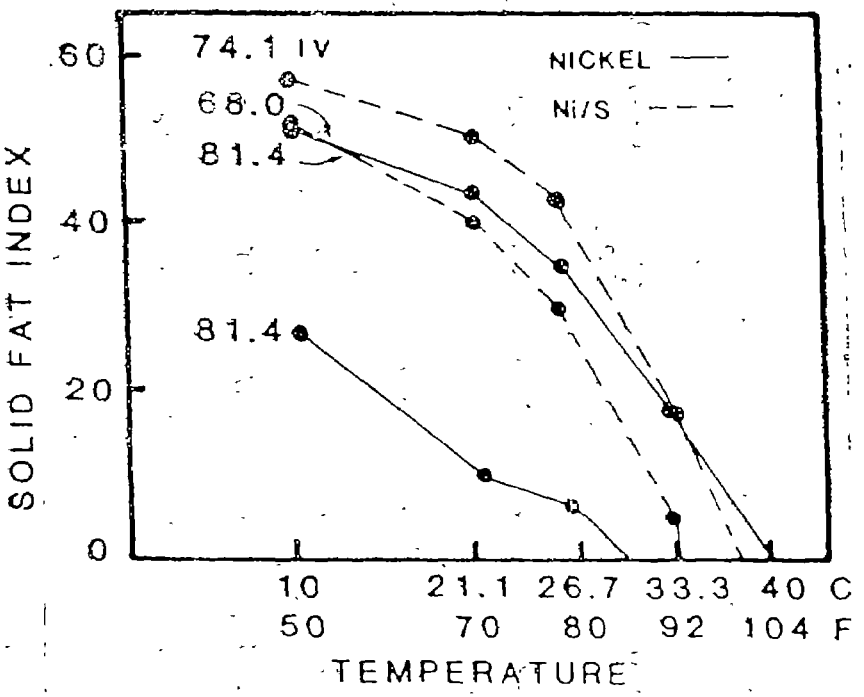

60
40
20
0
SOLID FAT INDEX
74.1 IV
68.0
81.4
81.4
NICKEL
Ni/S
10
50
21.1
70
26.7
80
33.3
92
40 C
104 F
TEMPERATURE

FIG. 2

TABLE IX Slip point and solid fat index of some interesterified fat products [5, 6]

Product	Slip point °C	Solid fat index (ml / kg)					
		15°C	20°C	25°C	30°C	35°C	40°C
Randomised Sal + Cottonseed (3 : 7 w/w)	35	10.9	8.9	5.4	3.5	1.4	—
Directed 15°C / 12 hr.	38	15.6	14.0	11.3	9.0	5.7	2.8
Randomised Sal + Sunflower	38	16.3	13.3	9.2	6.4	4.6	3.0
Sal + Rice bran (1 : 1 w/w)	35.5	15.0	11.7	7.4	5.0	2.4	0.5
Randomised Sal + Soybean (6 : 4 w/w)	36.5	20.1	14.8	10.1	8.0	5.4	3.8

TABLE X Interesterified products from palm oil alone and in combination with liquid oils

Product	Slip point °C	Solid fat index (ml / kg)					
		15°C	20°C	25°C	30°C	35°C	40°C
Palm randomised	41	38.0	32.0	25.5	21.2	18.0	13.0
Palm + Soyabean (80 : 20) randomised	35.0	26.0	16.0	12.1	12.0	8.6	5.0
Directed 15°C / 2 hr.	37.1	27.0	22.7	16.7	14.0	9.6	6.0
20°C / 2 hr.	37.1	27.5	21 8	16.7	13.7	9.1	5.3
Vanaspati (hydrogenated)	37.0	30.4	25.1	16.0	10.0	5.1	.3
Palm + Rice bran randomised (8 : 2 w/w)	37.5	22.3	19.3	14.8	10.0	9.0	5.0
Palm + Rapeseed (8 : 2 w/w)	37.0	22.0	15.2	13.2	9.0	6.0	2.6
Directed 15°C / 2 hr.							

TABLE XI Solidification Behaviour of Blended Products obtained from Sal Stearin with other Fat Fractions (Stearin/Mid).

Sample	Super cooling temperature °C/time in min.	Solidification temperature °C/time in min.	Rise °C
Sal Stearin + Palm Mid			
90 : 10	21.5/21	28 85/29	7 35
80 : 20	22.05/18	27.7/28	5.65
Sal Stearin + Mango Stearin			
85 : 15	21.65/19	30.05/32	8.4
70 : 30	21.8/18	31.6/31	9.8
Malyasian Cocoabutter	23.15/10	29.4/18	6 25

Slip point °C	SFI AT °C				
	15	20	25	30	35
32 2	71.5	67.5	62.0	25.1	0.93
30 0	68.2	63.9	50.8	43 6	1.71
33 0	72.7	70.9	64.4	37.6	6.8
33.7	73.5	72.8	67.9	44.7	1.7
Malyasian Cocoabutter 30.5	79.8	74.5	46 8	13	0.5

Fractionation of fatty oils

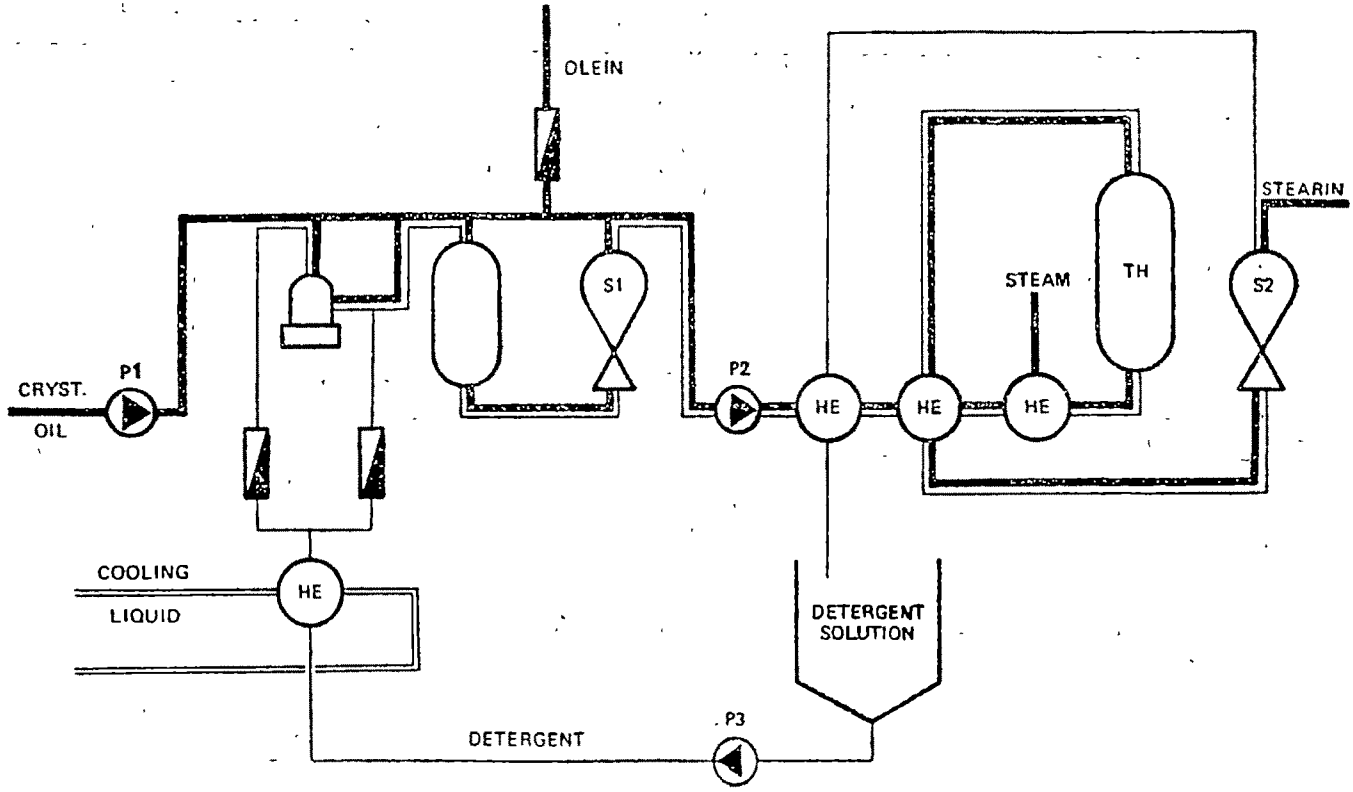


FIG. 3

TABLE XII Effect of amount of lipofrace agent and temperature on the extent of dewaxing of degummed* rice bran oil

Free fatty acid (%) in crude rice bran oil	Dewaxing condition				Wax removed %	Average Unsap. matter %
	Surface active compound		Mixing Temp. °C	Mixing Time hr.		
	Name	Amount (g/100 gm. oil)				
	Lauryl sulphate	0.5,1	20±1	3	76.0—88.0	—
			15±1	„	80.6—84.4	1.9
			10±1	„	83.4—86.8	—
15.3	Sodium oleate	0.5,1	20±1	„	75.2—77.2	—
			15±1	„	78.7—82.0	2.2
			10±1	„	82.0—84.0	—
	Alkylated phenol	0.5,1	20±1	„	76.8—79.2	—
	ethylene oxide		15±1	„	82.1—85.6	1.8
	condensate		10±1	„	85.2—87.8	—

* degummed by 85% phosphoric acid (1 kg/tonne oil)

TABLE XIII Interesterification of cocoanut and olive oils (1 : 1, w/w)

Triglyceride fatty acids carbon no.	Starting mixture wt. %	Interesterified starting mixture oils treated with:	Oils % change from in interesterification
	C	Alkali metal	C. Cylindracae lipase
36—38	29.2	−14.0	−13.0
40—48	12.0	+48.9	+48.3
50—56	58.7	+34.8	−35.2

TABLE XIV Triglycerides formed by interesterification[a] of a mixture of olive oil (5 parts) and stearic acid (1 part) using Rhizopus delemar lipase as catalyst[a].

Fatty acid	Amount in olive oil			Amount in interesterified triglyceride		
	Total TG	2-Position	1 and 3 Positions	Total TG	2-Position	1 and 3 Positions
	(%)	(%)	(%)	(%)	(%)	(%)
16 : 0	16.6	3.5	23.2	13.7	3.2	18.9
16 : 1	1.8	1.3	2.0	1.6	1 6	1 6
18 : 0	2.0	1.0	2.5	15.6	0.7	23.0
18 : 1	66.8	72.0	64.2	56 6	72 2	48.8
18 : 2	12.8	22.2	8.1	12 6	22.3	7.7

[a]A mixture of olive oil (2.5g) and stearic acid (0.5g) dissolved in 60—80°C petroleum ether (6.0g) was stirred at 40°C for 24 hrs. with hydrated catalyst (250 mg) prepared from R, delemar lipase and kieselguhr.

TABLE XV Triglyceride formed by interesterification of a mixture of palm midfraction (10 parts) and stearic acid (0.5 parts) using A. niger lipase as catalyst[a]

Fatty acid	Amount in triglyceride	
	Palm midfraction (%)	Interesterified product (%)
14 : 0	0.7	0.7
16 : 0	57 0	37.0
18 : 0	6.0	28.9
18 : 1	31.8	30.2
18 : 2	3.6	3 5
20 : 0	0.2	0.2
Triglyceride species[b]		
SSS	5	13
POP	58	19
POST	13	32
STOSt	2	13
SOS	7	2
SLnS	9	7
SOO	4	11
Others	2	3

S—saturated fatty acid group, P—Palmitic, St—stearic, O—Oleic, Ln—Linolic

TABLE XVI Hydrolysis of 3.33 Millimoles of cocoanut oil at pH 5.4 with A. niger lipase solution (Palatase) at various temperatures.[a]

Reaction time (hr.)	Percentage hydrolysis at			
	25°C	34°C	43°C	52°C
1	38.7	26.8	33.6	19 4
2	39 4	43.4	45,9	25.7
4	61.3	59.1	56.8	35.5
8	77.8	74.2	70.4	38.0
16	99.1	91.5	84.1	48 9

CONCLUSION

I have tried in this series of lectures to highlight some of the scientific facts, new developments which have taken place and what might happen in future in India in the next 15 years. The reason is that next to cereals oilseeds constitute the most important item of agricultural commodities in India. They occupy 11 per cent of gross cropped area and contribute to about 10 per cent of the value of output from agriculture. I have given importance to vegetable oils because these constitute 85 per cent of fats for human consumption, the balance being derived from milk fat. The value of vegetable oils being produced now is of order of Rs. 4000 crores to which must be added the imported value of Rs. 1500 crores in 1984-85. The product value will be of the order (in various way) of Rs. 10,000 crores which might double in the next 15 years. We can hardly afford to ignore the economic aspect of this commodity in agriculture and industry.

I do hope that the next decade will see in India much work related to both fundamental and applied sciences. Late Acharya Jagadish Chandra Bose through his example remains a pathbreaker and a 'GURU' in the real sense. Let us have the courage to imbibe his teachings which will be really the best way of paying homage to this great man.

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